Structure of the Actin Binding Protein (ABP1) from *Saccharomyces Cervisiae*

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Abstract No. almo0163
Beamline(s): **X9B**

In yeast, ABP1 and CAP co-localize to cortical actin patches, which are structures that target to regions of active cell growth. ABP1 also appears to be involved in dynamic cytoskeletal events in mammalian systems, as the murine homolog (mABP1) accumulates in lamellipodia of motile cells, and localizes specifically to sites enriched in the machinery that stimulate actin filament assembly. In addition to the C-terminal SH3 domain that binds CAP, ABP1 contains an N-terminal cofilin homology domain (CHD; 141 amino acids) that shows 23% identity with yeast cofilin, and which forms part of the F-actin binding site. Two hybrid data indicate that the N- and C-terminal domains of CAP physically interact, even though the functions of the N- and C-termini are not coupled. Interestingly however, the N-terminal domain of CAP appears to regulate the ability of the proline-rich linker region to bind SH3 domains, and may therefore be involved in sub-cellular localization. The mechanism describing the interactions between the N- and C-terminal domains, and the influence of these interactions have on ABP1 binding and localization remains an open question.

We have successfully used a six dimensional real space search to solve the structure of the N-terminal 140 amino acid domain from yeast ABP1 that has ~20% sequence identity with cofilin. This domain crystallizes in the monoclinic space group C2 (a=159.4, b=66.8, c=127.6, β=106.9°. These parameters indicated a large number of molecules occupied the asymmetric unit. The final structure contains 9 independent copies, which form 5 independent dimers with similar interfaces (four complete dimers in the ASU, and another dimer sitting around a crystallographic two-fold axis). Analytical ultra centrifugation studies are consistent with a dimer; however, gel filtration studies only show a monomeric species. Additionally, a significant portion of the actin-binding surface identified in cofilin is buried at the dimer interface, suggesting that the formation of the dimer interface must be regulated, or the actin binding surface differs significantly from that utilized in cofilin. There is a clear need to confirm the existence of this dimer, as it has important implications for the overall organization and function of the multi-protein complex to which ABP1 belongs.

As the structure solution was rather novel we are providing a more detailed description than normal. The weak homology (~20%) of the search model and the complex nature of the asymmetric unit is a common difficulty for MR and prevented the solution of ABP1 by conventional MR methods. We were able to obtain an EMP derivative, which due to non-isomorphism, was only useable to 6.0Å resolution. Phases based on the isomorphous and anomalous signals provided a poor SIRAS map that did not clearly identify molecular boundaries; however, the availability of even a poor experimental map allows for the use of the phased translation function {**if**} the initial orientation of the search model is known. As the rotation function did not yield an interpretable solution, a standard X-PLOR protocol for the phased translation function was modified to allow systematic sampling of all possible orientations for the search model in 100 increments along the three search angles. A total of 22,328 different orientations were used to calculate the phased translation function, requiring ~15 hours of CPU time on a single SGI ORIGIN200 processor, without optimization for space group symmetry. The realization of this six dimensional search is due to the fact that the phased translation function can be calculated via FFT. Filtering of the top scoring solutions resulted in eight independent solutions that were consistent with the self-rotation function and native Patterson. Phases were extended to 2.1Å resolution using eight-fold NCS averaging, which allowed for the identification of the ninth and final molecule in the asymmetric unit by difference Fourier synthesis. Further refinement resulted in an R-factor of 20.0% (R_{free}=29.1%).

These results suggest that the approach will have wide spread utility, as if even a modest search model is available, it will be possible to make significant progress towards a structure solution with a relatively poor map. We envision situations, where previously "uninterpretable" MAD- or MIR-derived maps (e.g., poor Se-Met phases) will be of significant value and will allow for the solution a number of previously intractable problems. This approach is likely to become increasingly important as crystallographers focus on multi-component complexes and as the representation of unique folds increases in the PDB. We are now trying to fully generalize this approach to systems for which a suitable MR search model cannot be identified. We are "re-formatting" the entire PDB, and specific common motifs (e.g., parallel β-sheets, anti-parallel β-sheets, etc.) as spherical harmonics so as to conveniently allow for the brute force search of "all structures" against an experimental map. The number of search models to be examined can be sensibly restricted by paying attention to the molecular weight and the secondary structure content (as estimated by circular dichroism) of the unknown structure. We strongly believe that this represents an area where significant automation and savings in hands-on effort can be realized, and these approaches may be particularly important for the many burgeoning structural genomics initiatives.